MINISTRY OF HOUSING AND LOCAL GOVERNMENT
REPORT OF COMMITTEE ON FISH TOXICITY TESTS

To the Right Honourable Anthony Greenwood, M.P.,
Minister of Housing and Local Government.

Sir,

We were appointed on 17th June, 1968, with the following terms of reference:

> "To consider the tests for toxicity to fish developed by the Ministry of Agriculture, Fisheries and Food and the Trent River Authority respectively and advise if either of them is suitable, or could be made suitable, for use in conjunction with consents to discharge effluents to streams."

1. We first met on 27th June, 1968, and in all have had three meetings.

2. Present-day legislation for the prevention of pollution of rivers stems largely from the Report of the Rivers Pollution Prevention Sub-Committee of the Central Advisory Water Committee (the Hobday Report) in 1949*. This paved the way for the Rivers (Prevention of Pollution) Act of 1951 which, together with the Act of 1961, provides for discharges to rivers of sewage and industrial effluents being subject to conditions relating to both volume and quality. To check the latter, testing or analysis is necessary.

3. The Hobday Committee recommended that river boards (as they then were) should all use the same tests for assessing the properties of a polluting liquid; this was obviously desirable, especially for empirical tests. In fact, a number of such tests had come into general use following the work of the Royal Commission on Sewage Disposal in the early years of the century and, because these had become well established and had secured general acceptance, it was only necessary to recommend formally that river boards should adopt them when formulating conditions of consent for effluents. For this purpose a small inter-departmental committee was formed and its report was issued with M.H.L.G. Circular No. 8/54, which recommended certain standardised tests. So far as is known, no question has ever been raised about the value of these recommendations.

4. In its report the inter-departmental committee drew attention to the fact that no standard fish toxicity test was in existence but hinted that one would probably be necessary. The Ministry of Agriculture, Fisheries and Food (M.A.F.F.), which was represented on that committee, was in fact then developing a fish toxicity test, partly for its own use but partly with the river boards' requirements in mind. The committee therefore continued to meet for some time in the hope that in due course it might be able to make a recommendation about that test. Eventually the test, which is reproduced as Appendix 1 to this Report, was standardised, and the committee believed that what was then necessary was to obtain some experience in the use of the test which would allow it, if the experience was suitably satisfactory, to recommend its general adoption. However,

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*H.M.S.O., 1949, reprinted 1959. Now out of print.

it was never able to do so, though M.A.F.F. has made much use of the test to obtain fundamental data for pesticides and other chemicals.

5. Meanwhile, control of pollution by river boards—now replaced by river authorities—continued, and much progress was made without a fish toxicity test. Clearly the lack of a specific toxicity measurement is no disadvantage provided it is known what the toxic material is; in such circumstances it is usually easier in practice to control the toxic ingredient rather than the toxicity of the effluent. This is the general practice which has been adopted. It has been facilitated by the discovery by the Water Pollution Research Laboratory that, in many instances, the toxicities of a number of substances in the same water can be added together to give roughly the toxicity of the whole.

6. Unfortunately, in some cases the constituents of an effluent are not known in any detail and it is not possible, therefore, to calculate its toxicity in that way. In other cases the difficulty of analysis of the offending constituents stands in the way of an assessment by this means. Also there are effluents which are very much more toxic, and some which are much less toxic, than would be expected from the known constituents. It is apparent, therefore, that the control of the toxicity of such effluents would be facilitated if a suitably simple test of sufficient accuracy were available by which a direct measurement could be made. Recently, the Trent River Authority, faced with the problem of being unable to identify the constituents responsible for the high level of toxicity of a particular effluent, developed its own toxicity test which, though simpler than that of the M.A.F.F., was not so accurate. The Trent River Authority believed the test would be adequate for its requirements and proposed to attach it to the consent conditions of the particular effluent. The discharger objected, and it appeared that there might be an appeal on the suitability of the test. On consideration, however, it was thought that the suitability of the test, being a general matter affecting ultimately all river authorities and perhaps many dischargers, ought not to be decided just in one local case, on the evidence of one river authority and of one objecting discharger. In the same way as a special committee had recommended the other general tests in use, it seemed consistent that a special committee should give an opinion upon this one. Moreover, since the test was new and experience of it necessarily limited both in time and scope, it was desirable that the committee should represent much wider interests than the original one. We were appointed with these matters in mind.

7. Our terms of reference are straightforward, simply requiring us to recommend whether or not either of the two tests is suitable, or could be made suitable, as a standard test for use in consent conditions. Although we find we are able to recommend the adoption of one of these methods, after modification, as a suitable standard test, we have nevertheless considerable reservations about the need for such a test other than in circumstances to be described later. We feel that we should make this plain, otherwise misunderstandings might arise. We have neither the right nor the desire to tell river authorities what kind of policy they should adopt, but we do believe that both they and the dischargers should know the lines of our thinking and that you, Sir, should know not only that we recommend a test as being suitable, but also that we recommend it to be used only sparingly.

The M.A.F.F. Test

8. As has already been mentioned, the M.A.F.F. developed a standard test several years ago. This test (Appendix 1) was designed to give accurate results in all foreseeable conditions. Accordingly, it provided against the loss of toxic material by volatilisation or oxidation during the test and also against changes brought about in the solutions by the fish themselves, for example by respiration and from excreta. To achieve this the toxic solution in contact with the fish had to be continuously renewed over the period (up to 48 hours) of the test; this involved the use of a large weight of material—and thus necessitated a large quantity of sample—in relation to the weight of fish being used for the test. To reduce the amount of sample, and so bring the test down in scale to something practicable, a very small fish had to be used. Since no British fish are sufficiently small all the year round, the choice fell on a foreign one, the harlequin (*Rasbora heteromorpha*); this fish appeared to have a sensitivity to toxic materials similar to that of trout, which is probably as sensitive or more sensitive than most of our own fish. Its small size and very low rate of consumption of oxygen compared with trout permit a very large reduction in the quantity of test solution required. It should be said, however, that difficulties are sometimes experienced in obtaining and keeping harlequin fish.

9. To meet these requirements the test vessel has to be provided with a means for continuously supplying the appropriate dilution of the toxic material over the period of the test at a controlled temperature and containing a sufficient concentration of dissolved oxygen. While such provisions certainly make for complexity, the test does give reproducible and reliable results; it has been widely used on pesticides and other organic materials, for which some fundamental and widely applicable data is in consequence now available. On occasions samples of industrial effluents are sent to the M.A.F.F. laboratory for testing on a repayment basis; the present charge is some £46 per test, a figure which is stated to be below the present economic cost. If it were the only test available we would be satisfied if one or two river authorities and perhaps one or two local authorities and industrial organisations installed the testing equipment for their own use so that the value of the test as one for controlling river pollution could be assessed in the field.

The Trent River Authority (T.R.A.) Test

10. This test is simpler than the M.A.F.F. test, but not so accurate or free from interferences. Though the same test procedure had always been followed as submitted to us few experimental details had been standardised. Since then we have standardised it, though no claim is made that the conditions laid down are the optimum ones. The matter is further dealt with in paragraph 17, and the standardised test is included as Appendix 2.

11. In the T.R.A. test no attempt is made to prevent the loss of volatile toxic material on the one hand, or the build-up of toxic matter in the solution due to the fish themselves on the other. Since the test solution is aerated but not renewed during the two-day period of the test, volatility, destruction, or absorption into the fish of toxic matter must introduce an error and the result will be that mortality is too low. The effect of any increase in toxicity caused by the activities of the fish will, of course, act in the opposite direction. Hence, the T.R.A. test

cannot be as precise as the M.A.F.F. test, and in some cases could give very misleading results. On the other hand, the apparatus used is very much simpler to construct and operate. Moreover, the test animal is trout which is readily available and acceptable. So far brown trout have been used, but since so much of our knowledge of toxicity relates to rainbow trout (*Salmo gairdnerii*) in both test and actual conditions, it has been agreed that rainbow trout shall be the test animal.

12. The whole question of choice of test therefore hinges on whether the greater accuracy and precision of the M.A.F.F. test is worth the extra cost and refinements. Or, put another way, does the T.R.A. test give results which are sufficiently precise for control purposes and for acceptance in a Court of Law? If the answer to this question is in the affirmative then, because of its simplicity, the standardised T.R.A. test should be used.

13. As we have seen, the essential difference between the two methods is that in the M.A.F.F. test, the test solution, containing an adequate supply of dissolved oxygen, is continually replaced, while in the T.R.A. test there is no replacement of the test solution, the dissolved oxygen requirements being satisfied by continuously bubbling a supply of air through the liquid. Continuous aeration in this way would only be objectionable if volatile poisons were present or if the stripping of carbon dioxide from solution by the air increased significantly the toxicity of any ammonia—some will be excreted by the fish—which might be present. On the question of volatile poisons, we accept that very few sewage effluents contain such materials likely to be lost in the sort of aeration that is given in the test. The possibilities of changes in toxicity during aeration have been examined by the Trent River Authority by introducing a fresh batch of fish into the test solution at the end of the 48-hour test period, and also by aerating the solution for 48 hours before testing; toxicities have then been compared with those observed in the "normal" test. Some ten different effluents have been tested in this way, and, with all but one, the results were very similar whether or not the sample had been aerated previously, indicating that in the majority of cases losses due to volatility and changes in toxicity were quite small. With the one exception, however, the effluent was apparently ten times less toxic after being aerated for 48 hours, indicating that some toxic substance had either been volatilised or oxidised during the aeration period and that the standard test would have given erroneous results.

14. A comparison of the two methods has been made by M.A.F.F. Using ten different effluent samples they found that the concentration giving a 50 per cent mortality was on average only about half as great with the T.R.A. test as with the M.A.F.F. test. There is no question, of course, of one being wrong and the other right. It is simply a comparison of two different tests, each empirical. Neither would there be any question of one test being more or less favourable to the river authority or the discharger; the permitted toxicity figure would be calculated to avoid that.

15. The same ten samples have been tested both in the laboratories of M.A.F.F. and those of T.R.A., each using the T.R.A. standarised method. Agreement was satisfactory. Also, reproducibility within each laboratory has been good.

16. On the basis of the experimental work we are satisfied that, in general, the T.R.A. test would be suitable, but that in a few isolated cases the test would be

quite misleading, giving results which are only a small fraction of the true figure. However, we do not consider those exceptional cases should condemn the test; after all, none of the empirical tests used for assessing the properties of a polluted liquid are universally applicable, but this has not prevented their useful application. Any empirical test has to be used with discrimination, and this we consider should be particularly so with this one. Indeed, it would be wise in each case where the use of the test was being considered to check that it will not give grossly misleading results. We therefore conclude that the T.R.A. test (suitably standardised) will be adequate for control purposes.

17. We have mentioned that the T.R.A. test as first submitted to us was not standardised, and pointed out the necessity of laying down precisely the conditions of empirical tests. The details given in Appendix 2 have been decided in agreement with the Trent River Authority and, if followed, should be adequate to enable different laboratories to obtain comparable results. It will be realised that some of the conditions laid down may be quite unimportant, but we do not know this and prefer therefore to make some decision about them.

18. The only point requiring further discussion is the hardness of the diluting water. It is known that the effect on fish of many poisons depends to a surprisingly large extent upon the hardness of the water, being usually though not invariably greater in soft water. The effect may also depend upon constituents more or less closely related to hardness, such as calcium, magnesium, chloride, sulphate, etc, and maybe on constituents unrelated to hardness. It is quite essential, therefore, that the composition of the diluting water, not merely its hardness, should be specified. It might be thought that water from the river to which the discharge is being made should be used, but this may well be variable and samples taken at different times might give different results which would be unacceptable. Just as some 20 years ago it was decided that the water for the B.O.D. test would have to be standardised and artificially prepared, so we believe should water for the toxicity test. However, because the considerable effect of hardness is known, and because hardness of rivers varies so much, we offer the choice of two—25 mg/l and 250 mg/l—so that one can be chosen which most closely approximates to the local conditions.

19. The provision of the standard diluting water will be expensive and often inconvenient. For routine purposes it may be shown that a result which is adequately accurate can be obtained with the use of a non-standard water—*e.g.*, a de-ionised water or even a tap water instead of a de-ionised distilled water containing added amounts of nutrient salts. We recognise it would sometimes be useful to allow such variations provided it was not claimed that the test was the one we recommend and provided it was not used for "formal" samples. Neither would we object to other departures from "standard" if used only for routine purposes, but would point out that results from standard tests could have a value in contributing to our knowledge of effluent toxicity which non-standard tests would not give. We do not therefore encourage non-standard tests.

20. We recommend that the duration of the tests should normally be two days. The general shape of toxicity curves (plotting median survival times against concentration) is such that a shorter standard period might be misleading, while a longer one would not give a result so much better than the two-day value as

to justify the extra time and effort needed. Exceptionally, it may be demonstrable that a period of three or four days might be highly desirable, and in these cases we would have no objection to its use. But additional trouble and expense without proven benefit is to be deprecated.

21. It must also be remembered that the period of test and the limiting toxicity laid down in a consent must be related to each other, and that to vary either without the other is to vary the severity of the condition. Thus, an effluent dilution which would kill 50 per cent fish in two days might kill all the fish in three or four days. If there was good reason for making the period three or four days it would be expected that a more dilute solution would be required for testing, and the dilution laid down as indicating toxicity would need to be raised.

22. It is seen from Appendix 2 that ten fish are used. For the purpose of inclusion in conditions of consent to discharge, it is suggested that the appropriate condition should be worded somewhat as follows:

> "When the effluent, after dilution to X times its volume with standard water of hardness 25 (or 250) mg/l, is tested by the method described in Appendix 2 of the Report issued with Circular ...., not more than five fish shall die in 48 hours."

23. If a local authority were the discharger of an effluent which was subject to a consent condition incorporating a limiting toxicity, this would normally be because it accepted toxic industrial effluents into its sewers and experience had shown that the effluent quality could not be adequately controlled otherwise than with such a condition. This might or might not mean that the local authority would be driven to incorporate conditions relating to toxicity in their consents under the Public Health Acts; this would depend upon the types of industrial effluent, whether they are broken down biologically and whether their toxicity individually can be assessed by chemical analysis. But it seems right to point out that our recommendation might mean that, sooner or later, some local authorities might have to use toxicity tests to control some discharges to their sewers. In addition, some local authorities and some industrialists subject to such a condition might want to be able both to check the results of others and to use the toxicity test in their own investigations.

24. We are not concerned at the moment with whether all these organisations arrange to carry out the tests themselves or use the facilities of others. This will depend on the number and frequency of tests they feel they will require. It should be mentioned that any laboratory which may be required to carry out tests at short notice must keep a supply of aerated diluting water and acclimatised fish in readiness, which may not be worth while for only a few tests per annum. Laboratories will also require a licence from the Home Office (see paragraph 30). More important in the present connection is the fact that in due course a number of organisations will be carrying out, and far more people of very different backgrounds will be trying to interpret, a test which is not only a new test but a very different kind of test from any which have been used in connection with effluents before. This could easily result in both misinterpretation and misapplication, and the following paragraphs are included in an attempt to avoid this happening.

25. The way in which we have suggested the condition should be worded (paragraph 22) is unusual, but it avoids having to define what "toxicity" is. The

same sort of expression could, of course, have been used in connection with any other test, had it been of any advantage in those cases. For instance, a requirement for a B.O.D. not exceeding 20 mg/l could have been laid down in the terms "When the effluent, after dilution to four times its volume with standard water, is tested by the method for B.O.D. described in the appropriate publication, not more than 5 mg/l of oxygen shall be consumed in five days". But this is neither necessary nor advantageous, because the amount of oxygen consumed is proportional to the B.O.D. and in fact becomes the B.O.D. when multiplied by the dilution factor. With the fish toxicity test, however, it cannot be said that a solution which causes eight out of ten fish to die is twice as toxic as one which causes four fish to die. It may be that something half as toxic as one which causes eight fish to die will not cause any at all to die, and that something twice as toxic as that which causes four to die will cause every fish to die. Moreover, whether four or six (or perhaps even three or seven) fish die in a given time may be little more than a matter of chance, whereas a variation of 50 or 100 per cent in a B.O.D. result would (in experienced hands) undoubtedly be real and meaningful. Because of this great care should be taken in interpreting a single test. If it is desired to know whether a solution just fails to reach, or just succeeds in reaching, the standard, or does so by a very wide margin, it will be necessary to carry out the test at dilutions other than X, and we believe that this should often be done.

26. As has been mentioned, we are of the opinion that for formal control purposes the test should be specified sparingly and with discrimination. In probably well over 90 per cent of all cases, river pollution control has been satisfactorily effected in spite of the lack of such a test, and in those circumstances we would certainly not recommend that one should be incorporated in conditions of consent now. There is no point in laying down conditions which are quite unnecessary.

27. In the case of sewage effluents which are known to be almost entirely derived from domestic premises, for instance, there would seem to be no case at all for having a toxicity condition. Similarly, if an organic effluent, such as one from a maltings or a cider factory, were of such a nature that satisfied the normal requirements such as suspended solids content and B.O.D., then provided adequate dilution were afforded it would not be toxic and there would be no need for a toxicity condition. In the case of effluents containing known toxic constituents, for example, phenols, cyanides and metals, then in so far as toxicity is at present controlled by limiting the concentrations of these constituents independently, this method of control should be continued and there would be no need for a toxicity condition. However, in respect of a complicated effluent of this nature, and perhaps of an effluent from a sewage treatment works with a variety of toxic materials admitted to the sewer, it might be to the advantage of both sides to substitute a toxicity condition for a whole series of individual conditions controlling particular constituents. In such a case it would be in order for an investigation to be made by the river authority and the discharger to see if this was so. If the toxicity test was found to be the better method to use for controlling the discharge, then there would appear to be no reason why it should not be adopted and why it should not be acceptable. Finally, there would appear to be no purpose in a toxicity condition if the survival of fish in the receiving stream is of no importance.

28. Without doubt the main application of the toxicity test would appear to be in cases of effluents likely to be toxic but where the nature of the toxic constituents are either not known or cannot be determined readily by ordinary analysis. It is true that it may be necessary to carry out, where it is possible, the complicated analysis in an attempt to obtain information on the best way of treating the particular waste, but this type of analysis does not seem to be suitable to apply to conditions of consent. In such cases it would be simpler to use a toxicity test, and this we would recommend.

29. In the light of experience it may become apparent that the test is of either more or less value and simpler to apply than we now think; in the event of such a happening we would certainly advocate a change in policy. Likewise, if it became possible to improve the test to give more accurate and reliable results, then clearly we would want a revision to be made—in the same way that the chemical tests for sewage and effluents are at present being revised.

30. It may be useful to point out that it would appear to be necessary for any laboratory where it is proposed to carry out fish toxicity tests to be registered under the Cruelty to Animals Act 1876, and for operators to be licensed under that Act. Applications should be made to: The Under Secretary of State, Home Office, Romney House, Marsham Street, London S.W.1.

Recommendation

31. Our recommendation is given below. The use of the headings (*a*)–(*g*) is for convenience only; they are not separate recommendations, and any quotation of one without the others would be a distortion of our views.

(*a*) The test devised by the Trent River Authority and standardised as in Appendix 2 is suitable for use in river authority conditions of consent to discharge, and if a fish toxicity test is needed for that purpose this one should be specified.

(*b*) A fish toxicity test is only rarely required, and in particular should not be used in connection with:

  (1) discharges which experience has shown are effectively controlled without it;
  (2) discharges derived from domestic sewage or other polluted water known not to be toxic at concentrations permissible on other grounds;
  (3) discharges where the toxicity is accounted for by one or two constituents which can readily be determined; it is then simpler to limit the concentration of these constituents;
  (4) discharges into waters in which survival of fish, at the point of discharge or downstream, neither is nor is likely to become of any importance.

(*c*) It follows that the test will find its chief value in relation to discharges of considerable importance, the toxicity of which is very different from that which can be accounted for by ordinary chemical analysis or is difficult to assess by that means.

(*d*) Because the relationship between the effect of a discharge on fish life and the permitted value of the 2-day toxicity is not accurately known and may be variable, it is essential that knowledge and experience of it should be built up. The use of a toxicity test in a consent should be primarily to permit of this being done and only secondarily to provide for prosecution should this be necessary.

(*e*) Although we think the test is sufficiently accurate, its very nature must mean that there is a certain variability in the results. We feel sure that river authorities can be relied upon not to penalise a discharger because of failure to reach the standard by an amount which could easily be due to chance.

(*f*) We accept that if a river authority restricts fish toxicity in an effluent from a local authority works it may sometimes be necessary for the local authority to add related conditions to their consents to discharge some industrial effluents to their sewers. They too may sometimes need facilities for toxicity testing.

(*g*) We do not suppose we have standardised for ever. If major improvements in the test ultimately become available then they should be adopted according to a consensus of opinion at that time.

We have the honour to be

Sir,

Your obedient Servants

(Signed) A. KEY (*Chairman*)
J. S. ALABASTER
I. R. H. ALLAN
E. A. B. BIRSE
P. N. J. CHIPPERFIELD
R. W. EDWARDS
H. FISH
C. J. JACKSON
A. V. HOLDEN
S. H. JENKINS
W. F. LESTER

G. A. TRUESDALE
Secretary
August 1969

APPENDIX 1

Specification for the M.A.F.F. Fish Toxicity Test

(for use in Consent Conditions)

Definition of toxicity

The toxicity of an effluent shall be defined as the number of volumes of water to which one volume of effluent has to be diluted by addition of standard water in order that 50 per cent of a batch of 10 Harlequin fish (*Rasbora heteromorpha* Dunker) shall survive for 48 hours at a temperature of 20°C. under the conditions specified here.

Apparatus

Glazed aquaria shall be used for holding stocks of test fish and arrangements shall be made to maintain them at a temperature of 20°C. ± 1°C. The water in the aquaria shall be aerated and in continuous flow.

500 ml spherical short-necked glass flasks with ground glass B 24 sockets (B.S.572) shall be used for containing the fish in dilutions of effluent. Into the socket shall be fitted a gas wash-bottle head with a ground glass cone joint (B.S.572). The end of the down tube of the wash-bottle head shall be 15 to 25 mm from the bottom of the flask, and shall be connected at its upper end by tubing of 5 mm bore to dosing apparatus which shall deliver 100 ml of test solution to the test vessel every 10 minutes.

The arrangement of dosing unit and test vessel which is recommended is shown diagrammatically in Fig. 1.

The dosing apparatus shall consist of a 105 ml bulb burette (B.S.846) inverted so that the bulb is at the bottom and having the ends cut off leaving 1 to 2 cm of plain tubing beyond the 105 ml and zero graduation marks. The lower end shall fit flush to a piece of 10 mm bore tubing—about 50 mm long—the joint being covered by a sleeve of vinyl tubing. Across the lumen of this piece of tubing a sintered glass plate of porosity grade 4 (B.S.1752) shall be fused so that the distance between its upper surface and the zero graduation mark on the burette is not greater than 70 mm. The tube below the sintered glass plate shall be connected to a supply of air. Three glass side arms of 3 mm bore and 10 to 20 mm long shall be inserted and fused into the wall of the tube above the sintered glass plate, one to be connected to a supply of dilution water, the second to a supply of effluent and the third to the down tube of the wash bottle head on the test flask. These three connections shall be of glass and/or vinyl tubing 3 to 5 mm in bore. The supply of dilution water shall be at a constant level which shall be adjustable within the range of the level of the 100 ml and zero graduation marks on the burette. The supply of effluent shall be from a vessel in which the level is kept to that of the 100 ml graduation mark on the burette. The length of the connection between the effluent constant level vessel and the burette shall not exceed 2 m. The volume of liquid in the constant head vessel shall not exceed 25 ml, the surface area of liquid exposed to the atmosphere shall not exceed 600 mm^2 and the volume of liquid passing through the vessel shall not be less than 300 ml per hour. The connection between the burette and the wash bottle head shall not exceed 300 mm in length.

Provision shall be made for closing and opening the four tubes which are connected to the burette, so that each one opens in turn once every 10 minutes while the other three remain closed. The tube connecting the burette with the diluting water constant head vessel shall open for 1 minute to allow the required volume of dilution water to enter the burette; next in the cycle the tube connected to the compressed air supply shall open for 3 minutes to aerate the dilution water; then the tube connecting the burette with the effluent constant head vessel shall open for 1 minute to allow the required volume of effluent to enter the burette and bring the level of liquid up to the 100 ml graduation mark; finally at the end of the cycle the tube connecting the burette with the test vessel shall open for 1 minute to allow the 100 ml of test solution to drain into the test vessel. The overflow level from the test vessel shall be arranged so that it is level with the zero graduation mark of the burette.

A minimum number of 2 test vessels shall be used for testing any one sample of effluent, one being for the dilution of effluent specified in the consent condition and the other for dilution water alone for control purposes.

A single constant head vessel for effluent shall be common to all the burettes used and it shall be supplied from a 20-l glass aspirator by tubing 2 to 5 mm in bore and not more than 0·5 m in length. The aspirator shall be closed by a cotton wool plug.

Provision shall be made to aerate the dilution water before it reaches the constant level vessels to ensure that the dissolved oxygen concentration is from 90 to 100 per cent of the air saturation value.

The temperature of the test solution in the test vessels shall be maintained at 20°C. ± 0·5°.

Size of sample of effluent

The volume of effluent sample shall be related to the toxicity (dilution) specified in the consent conditions. It shall be at least 30 l (6½ gal) for a specified toxicity of 0 and shall be reduced in proportion to the increase in the specified toxicity; thus it shall be at least 3 litres for a toxicity of 10 and shall be at least 300 ml for a toxicity of 100.

Collection and treatment of sample of effluent

The sample of effluent shall be collected in glass or polythene containers which are completely filled and stoppered and despatched as quickly as possible to the testing laboratory. On arrival it shall be stored at a temperature of 0 to 4°C. until the test is started, but the period between the time the sample was taken and the time the test is started shall not exceed one week.

Preparation of water to be used for diluting effluents and for keeping stocks of fish

Water for preparing the test dilutions of effluents and for keeping the test fish before the tests shall be prepared by adding stock solutions of analytical reagents to de-ionised (or distilled) water whose conductivity does not exceed 10 conductivity units (i.e. 10 micromhos per cm^3).

Stock solution 1. Dissolve 320 gm $CaCl_2.6H_2O$, 29 g NaCl and 9 g $NaNO_3$, and make up to 1 litre.

Stock solution 2. Dissolve 151 g $MgSO_4.7H_2O$ and 79 g Na_2SO_4 and make up to 1 litre.

Stock solution 3. Dissolve 27·5 g $NaHCO_3$ and make up to 1 litre.

For each 100 litres of standard water to be prepared add 10 ml each of Solutions 1 and 2 and 100 ml of Solution 3.

Conditions under which fish shall be kept

Fish used for testing shall show no signs of disease and shall not have been used in previous tests, including control tests.

Fish used for testing shall be greater than 13 mm and less than 30 mm in length from the top if the snout to the fork of the tail.

Before the fish are used in a test they shall be kept for at least 1 week in the standard water and in standard conditions at a temperature of 20°C. ± 1°C. and with the concentration of dissolved oxygen maintained at a value between 80 and 100 per cent of the air saturation value by means of aeration with air. They shall be kept in glazed aquaria at a density not exceeding 5 per litre of water and shall be fed to repletion once a day (except on the day they are to be used for a test). The following have been found to be a suitable diet: live or dried daphnia, minced bullock's heart and wheat germ.

Test procedure

The 20-l aspirator vessel shall be filled with effluent or (if the effluent is of high toxicity) diluted effluent. If diluted effluent is used in the aspirator vessel then the concentration shall be not less than 5 times that of the dilution used in the fish test.

The test fish shall be taken in a net from the stock tanks and distributed at random into batches of 10 in 1 litre beakers which are half full of standard water. Each batch of fish shall be strained out into a net, washed back into the beaker using the appropriate test solution (or, in the case of controls, dilution water) taken from the test vessels, and then poured into the test vessel with the appropriate test solution or dilution water. The test vessels shall be supplied every 10 minutes with 100 ml of test solution or in the case of the control, dilution water. Dead fish shall be removed after 24 hours and again at the end of the 48 hour test period when the total mortality shall be recorded. If during the test there are any deaths in the batch of control fish the test shall be invalid.

APPENDIX 2

Recommended Standard Fish Toxicity Test

Definition of Toxicity

The toxicity of an effluent may be defined as the number of volumes to which one volume of a sample must be diluted with standard water in order that 50 per cent of a batch of ten rainbow trout (*Salmo gairdnerii*) will survive for 48 hours at a temperature of 15°C. under the conditions of the test specified below. However, it is not necessary to use the definition in a consent condition (see paragraph 22 of Report).

Test Fish

The test fish, rainbow trout (*Salmo gairdnerii*) should be between 60 and 150 mm in length and should not show signs of disease nor have been used in previous tests (including controls).

Test Fish Storage and Acclimatisation

Stocks of test fish should be kept in aquaria maintained at 15 ± 1°C. and aerated to provide adequate oxygen and mixing. The inside of the aquaria should not consist of, nor be protected by, material which could yield up any toxic matter to the water. The water need not be standard dilution water but should approximate in composition (particularly as regards hardness) to it. If it does, then two days acclimatisation in standard water before a test would be adequate; if it does not, five days should be allowed.

Apparatus

(1) At least two aquaria made of perspex or other suitable material and measuring approximately 600 mm × 300 mm × 300 mm deep (24 in. × 12 in. × 12 in.), each provided with one standard 1 in. (25 mm) cube diffuser stone for aeration.

(2) Facilities for temperature control of aquaria at 15 ± 1°C.; this control may be achieved by the use, for example, of a temperature-controlled room or thermostatically-controlled coils.

(3) An aeration pump capable of aerating each aquarium at a rate of at least 50 ml air per minute.

(4) Glass or polythene containers for collection of samples.

Dilution Water

One of two standard waters of total hardness 25 or 250 mg/l expressed as calcium carbonate is required. This water is prepared from de-ionised distilled water of conductivity not exceeding 10 micromhos, by the addition of appropriate quantities of the following stock solutions of mineral salts:

Stock Solution 1. Dissolve 400 g $CaCl_2.6H_2O$, 36 g NaCl and 11 g $NaNO_3$ in de-ionised distilled water and make up to 1 litre.

Stock Solution 2. Dissolve 189 g $MgSO_4.7H_2O$ and 99 g Na_2SO_4 in de-ionised distilled water and make up to 1 litre.

Stock Solution 3. Dissolve 34 g $NaHCO_3$ in de-ionised distilled water and make up to 1 litre.

For standard water of 25 mg/l hardness add 10 ml each of Solutions 1 and 2 and 100 ml of Solution 3 to 100 l of de-ionised water. For standard water of hardness 250 mg/l add 100 ml each of Solutions 1 and 2 and 1 litre of Solution 3 to 100 l of de-ionised water.

Effluent Sample

The volume of effluent sample required is, of course, related to the dilution specified in the condition. Since the total volume used in the test is 20 l (4½ gal) the volume of sample is 20 l divided by X in the consent condition (see paragraph 22).

The sample should be collected in glass or polythene containers which are completely filled and stoppered. The test should be started as soon as possible after collection of the sample, but in any case within 24 hours.

Procedure

To one aquarium is added the appropriate volume of effluent, suitably diluted (where necessary) to a total volume of 20 l with whichever of the two standard dilution waters is specified. To another is added 20 l of standard dilution water only. The temperature is controlled at 15 ± 1°C.

The aquaria are aerated by means of the diffusers until the dissolved oxgyen content of the water in them is in excess of 5 mg/l. Ten rainbow trout (acclimatised as described above) are selected at random and placed in each tank, where they are retained for 48 hours. During that time aeration is continued at a rate not greatly above the minimum required to maintain the dissolved oxygen in excess of 5 mg/l. This rate may be no more than 50 ml/min. but this depends upon the characteristics of the particular diffusers and the B.O.D. of the solution. The frequency with which the dissolved oxgyen needs to be checked depends upon the experience of the operator with the diffusers in use and the effluent under test.

After 48 hours the number of dead fish in the test solution is counted. If during that time there are any deaths in the batch of control fish, the test is invalidated.

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MINISTRY OF HOUSING AND
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Fish Toxicity Tests

Report of the Technical Committee

5 MR
1970

LONDON
HER MAJESTY'S STATIONERY OFFICE
1969

The Technical Committee to advise on Fish Toxicity Tests was appointed by the Minister of Housing and Local Government on 17th June, 1968, with the following terms of reference:

> "to consider the tests for toxicity to fish developed by the Ministry of Agriculture, Fisheries and Food and the Trent River Authority respectively and advise if either of them is suitable, or could be made suitable, for use in conjunction with consents to discharge effluents to streams."

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